SDS-PAGE Protocol

Preparing samples

1. Spin down cultures at 5,000 g for 10 minutes and pour off supernatant.
2. Re-suspend in the leftover liquid and measure the volume of the suspension. Add 5x loading buffer, diluting the buffer to 1x.
3. Transfer mixture to 1.5 or 2 mL tubes and heat at 80^{o}C for 20 minutes.

Making the gel

1. Make two separate tubes of running and stacking gel according to the formula detailed below. *Do not add TEMED yet
2. Clamp a 1mm glass plate with the appropriate glass cover together with the green clamps, making sure the plates are flat against the table. 2 plates can be made and run at a time.
3. Place the clamped plates into the clear stand, making sure the bottom of the plates are pressing into the rubber strip.
4. Add 10mL TEMED to the running gel mix, swirl, and quickly pipet the mixture into the plates by placing the tip against the crack between the two plates. Fill to the bottom of the green stripe.
5. Immediately but gently, pipette water into the plates, being careful not to disturb the gel underneath. Fill to the top of the plates.
6. Wait 5-10 minutes, or until the gel appears to have set. Use the leftover running mix to determine if the gel is ready or not
7. Once the gel is set, pour off the water and re-clamp the plates. Add 5mL TEMED into the stacking gel mix, swirl, and pipette into the plates. Fill to the top of the green bar.
8. Place a 1mm well comb into the plate and wipe away any excess gel mix that leaks out of the top.
9. Wait 10-20 minutes (the stacking gel will take longer to set) until the excess stacking gel mix has hardened, then carefully remove the comb.

   NOTE: If there is only a very small amount of gel mix leftover (<2ml) then the leftover mix might not harden. If it hasn't hardened after 20 minutes, slowly lift comb to check for setting. If you see bubbles or liquid rush into the wells, gently place the comb back down and wait another 10 minutes. If the wells hold their shape, the gel has hardened.

Running the gel

1. Press the your plates, shorter plate side facing inward, against the middle section of the running box and slowly slide them into the holding case (make sure the clamps on the case are loose), then clamp in place. If you only made one gel, use another glass plate (specifically for taking the place of another gel) on the other side.
2. Place the entire case into the running box, making sure it's completely inside of the box. Pour in running buffer.
   NOTE: Fill the middle section with running buffer, and check for leaks. If the buffer leaks from the bottom into the box, then pour running buffer into the entire box, making sure to cover the plates. If not, you do not have to pour buffer into the entire box.
3. Load ladder and samples into the desired wells.

4. Run at 180V for 60 minutes.

Gel visualization

1. Remove the gel from the glass plates by carefully peeling off the top plate, and then gently pulling the gel off.
2. Soak the gel in staining buffer for 30 minutes.
3. Transfer the gel into a different container and soak in destaining buffer overnight. The gel should have visible bands the next day.

Gel Mix and Buffer Formulas

	Stacking Gel (makes 5mL)	15% Running Gel (makes 10mL)
ddH_2O	2.975 mL	2.2 mL
TrisHCl	1.25 mL (0.5M)	2.6 mL (1.5M)
10%(w/v) SDS	50 μL	100 μL
30% Acrylamide/ 0.8%w/v Bis-a	670 μL	5 mL
10% (w/v) A. Persulfate	50 μL	100 μL
TEMED	5 μL	10 μL

Loading buffer: (for 10 mL)

SDS-PAGE	1 g
β-mercaptoethanol	7 μL
Glycerol	2 mL
2M Tris pH6.8	1 mL
Bromophenol Blue	0.05 g

Running buffer*: (for 300 mL)

25 mM Tris	3.75 mL
200 mM Glycine	4.5 g
0.1% w/v SDS	0.3 g

Staining buffer*: (for 1000 mL)

Coomassie Blue	1 g
Acetic acid	100 mL
Methanol	400 mL
ddH_2O	500 mL

Destaining buffer:

Methanol	200 mL
Acetic Acid	100 mL
ddH_2O	700 mL

*Running buffer and staining buffer are reusable. Pour back into original container after you are done with them.